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(c) encoding a polypeptide as set forth in SEQ ID NO: 5 with at least one amino acid deletion, wherein the encoded polypeptide, upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation;

(d) encoding a polypeptide as set forth in SEQ ID NO: 5 which has a C- and/or N-terminal truncation, wherein the encoded polypeptide, upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation;

(e) encoding a polypeptide as set forth in SEQ ID NO: 5 with at least one modification that is a conservative amino acid substitution, an amino acid insertion, an amino acid deletion, C-terminal truncation, or N-terminal truncation, wherein the encoded polypeptide, upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation;

(f) of any of (a) - (e) comprising a fragment of at least about 16 nucleotides;

(g) that hybridizes to the complement of the nucleotide sequence of any of (a) - (f) under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences;
or

(h) complementary to the nucleotide sequence of any of (a) - (g).

REMARKS

Claims 1-3, as amended, and claims 4-8, 10, 11, and 43-45, as filed, are pending in the instant application. Claims 9, 12-42, and 46-56 have been canceled without prejudice or disclaimer. Support for the amendments to the claims can be found in the specification at page 18, lines 13-14 and page 103, lines 19-27. No new matter has been added as a result of the above-described amendments. The rejections set forth in the Office Action have been overcome by amendment or are traversed by argument below.

1. Rejections of claims 1-8, 10, 11, and 43-45 under 35 U.S.C. § 112, first paragraph

The instant Office Action maintains the rejection of claims 1-8, 10, 11, and 43-45 under 35 U.S.C. § 112, first paragraph, as lacking enablement commensurate with the scope of the claims. The Office Action mailed August 24, 2001 asserted a rejection of claims 1-8, 10, 11, and 43-45 as not being enabling for variants, sequences identified by hybridization, and fragments of the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 4. The prior Action stated that no

particular biological activity for IFN-L is set forth in the specification, and therefore, that one of ordinary skill in the art would not be able to make nucleic acid fragments or variants encoding polypeptides possessing the same activity as the claimed invention. The instant Action states that the claimed IFN-L variants are not limited to any particular activity and that the specification contains no definition of "an activity of the polypeptide set forth in SEQ ID NO: 5." The instant Action asserts that, in the absence of guidance as to a particular functional activity or structural elements required for function, it would require undue experimentation for one of ordinary skill in the art to make and use the invention as claimed. The instant Action also states that because tyrosine phosphorylation of cellular proteins occurs in response to many different stimuli, the requirement that the claimed IFN-L polypeptides possess "phosphorylation activity" would not provide any particular functional limitation.

Applicants contend, contrary to the assertion made in the Action mailed August 24, 2001, that the specification sets forth a particular biological activity for IFN-L polypeptides at page 103, lines 19-27, in an Example entitled "Biological Activity of IFN-L Polypeptides." Specifically, the specification discloses that when several cell lines were exposed to a rat IFN-L-Fc fusion polypeptide, an increase in cellular protein tyrosine phosphorylation was detected.

Applicants also contend, contrary to the assertion made in the instant Action, that the requirement that the claimed IFN-L polypeptides cause an increase in cellular protein tyrosine phosphorylation provides a particular functional limitation. Interferons are known to activate gene transcription by acting through a signaling pathway involving cell-surface receptors, tyrosine kinases, and cytoplasmic transcription factors (Shuai, 1994, *Curr. Opin. Cell Biol.* 6:253-59; Gilmour *et al.*, 1995, *Gene Expr.* 5:1-18; Larner *et al.*, 1996, *Biotherapy* 8:175-81). For example, in the IFN- γ signaling pathway, IFN- γ first binds the IFN- γ receptor, resulting in the phosphorylation of two members of the Janus tyrosine kinase (JAK) family, Jak1 and Jak2 (Pestka, 1997, *Semin. Oncol.* 24(3 Suppl. 9):S9-18-S9-40). The JAK kinases, in turn, phosphorylate the IFN- γ receptor, which then serves as a recruitment site for a member of the signal transducers and activators of transcription (STAT) family, Stat1 α (Pestka, 1997). Following recruitment, Stat1 α is phosphorylated and then released as an active transcription factor for IFN- γ -induced genes (Pestka, 1997). Analogous signaling pathways have been delineated for IFN- α , IFN- β , and IFN- ω , wherein the binding of interferon to a cell-surface receptor leads to the activation of JAK tyrosine kinases and the

subsequent phosphorylation of STAT cytoplasmic transcription factors (Gilmour *et al.*, 1995; Larner *et al.*, 1996; Pestka, 1997; Heim, 1999, *J. Recept. Signal Transduct. Res.* 19:75-120). The critical role played by protein phosphorylation in interferon signaling, therefore, is well established in the art (Williams *et al.*, 1997, *Semin. Oncol.* 24(3 Suppl. 9):S9-70-S9-77; Stark *et al.*, 1998, *Annu. Rev. Biochem.* 67:227-64). Applicants contend that because the stimulation of protein tyrosine phosphorylation is a well-established functional characteristic and functional property of interferons, the requirement that the claimed IFN-L polypeptides cause an increase in cellular protein tyrosine phosphorylation provides a particular functional limitation that would permit one having ordinary skill in the art to make and recognize nucleic acid fragments or variants encoding polypeptides possessing the same activity as the claimed invention.

In view of the knowledge in the art that interferons activate cellular protein tyrosine phosphorylation, and further in view of the disclosure in the instant specification that an IFN-L-Fc fusion polypeptide activated cellular protein tyrosine phosphorylation, Applicants contend that one of ordinary skill in the art would readily recognize that one "activity of the polypeptide set forth in SEQ ID NO: 5" is to cause an increase in cellular protein tyrosine phosphorylation. Nevertheless, in an effort to expedite prosecution, Applicants have amended claims 1-3 to replace the phrase "wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 5," with the phrase "wherein the encoded polypeptide, upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation." Applicants contend that the claims, as amended, are directed to IFN-L variants having a characteristic interferon activity, and that their specification teaches the skilled artisan how to make and use the claimed invention without undue experimentation. Withdrawal of this ground of rejection is therefore respectfully solicited.

The instant Office Action also maintains the rejection of claims 1-8, 10, 11, and 43-45 under 35 U.S.C. § 112, first paragraph, as lacking written description. The Office Action mailed August 24, 2001 asserted a rejection of claims 1-8, 10, 11, and 43-45 as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The prior Action stated that the disclosure of two nucleic acid sequences does not adequately describe the scope of the claimed genus, which encompasses a substantial variety of subgenera. The prior Action noted that a description of a genus of cDNAs may be achieved by means of a recitation

of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus *or* a recitation of structural *or* functional features common to the members of the genus. The instant Action states that the claims are drawn to molecules having “an activity of the polypeptide set forth in SEQ ID NO: 5,” rather than a phosphorylation activity and that the specification contains no definition of phosphorylation activity. The instant Action also states that because phosphorylation is not specific to any particular molecule or class of molecules, phosphorylation is not an activity that defines a genus of IFN-L molecules.

As discussed above, it is well established in the art that tyrosine protein phosphorylation plays a well-recognized role in interferon signaling; in fact, stimulation of protein tyrosine phosphorylation is a characteristic functional property of interferons. Applicants contend that the specification clearly discloses that the IFN-L polypeptides of the invention possess this characteristic functional property (at page 103, line 25). To expedite prosecution of the instant application, Applicants have amended claims 1-3 to recite that the claimed IFN-L polypeptides of the invention “upon exposure to mammalian cells, cause[] an increase in cellular protein tyrosine phosphorylation.” Applicants contend that because the amended claims recite a characteristic functional feature common to the members of the genus of claimed IFN-L polypeptides, the claims satisfy the written description requirement of 35 U.S.C. § 112, first paragraph. Withdrawal of this ground of rejection is therefore respectfully solicited.

Applicants respectfully contend that rejections based on 35 U.S.C. § 112, first paragraph, have been overcome by amendment or traversed by argument, and request that the Examiner withdraw all rejections made on this basis.

2. Rejections of claims 1-8, 10, 11, and 43-45 under 35 U.S.C. § 112, second paragraph

The instant Office Action maintains the rejection of claims 1-8, 10, 11, and 43-45 under 35 U.S.C. § 112, second paragraph, as being indefinite in the recitation of hybridization conditions. The Office Action mailed August 24, 2001 stated that claims 1-8, 10, 11, and 43-45 are indefinite because they encompass molecules identified by hybridization and hybridization conditions are not defined in the specification. The instant Action states that because the specification contains no limitations on what is considered stringent or moderately stringent, one of ordinary skill in the art would not be able to determine what conditions, and thus what molecules, were encompassed by the

claims.

Applicants have amended claims 1-3 to encompass molecules identified by hybridization “under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences.” Applicants contend that the skilled artisan could use the teachings of the specification and knowledge in the art to determine hybridization conditions in which “no more than a 21% mismatch between the nucleotide sequences” was obtained. For example, the specification discloses that when hybridization is performed at 50°C in a buffer containing 0.015 M Na⁺, such results can be obtained. Applicants also contend that one of ordinary skill in the art would appreciate that such results can be obtained using higher temperatures and lower Na⁺ concentrations, provided that a denaturing agent (such as formamide) is added to the hybridization buffer. *See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual* 6.59-6.60 (3rd ed. 2001) (describing an equation for determining the melting temperature of duplex DNA in a hybridization buffer containing formamide), a copy of which is enclosed. Applicants contend that the amended claims satisfy the requirements of 35 U.S.C. § 112, second paragraph, and therefore, respectfully request withdrawal of this rejection.

The instant Office Action also maintains the rejection of claims 2-8, 10, 11, and 43-45 under 35 U.S.C. § 112, second paragraph, as being indefinite in the recitation of “an activity of the polypeptide set forth in SEQ ID NO: 5.” The instant Action states that because the specification does not provide a limiting definition of “an activity of the polypeptide set forth in SEQ ID NO: 5,” one of ordinary skill in the art would not know what molecules were encompassed by the claims. Moreover, the instant Action notes that limitation of the claimed polypeptides to those having phosphorylation activity would not overcome the rejection, as there is no definition of phosphorylation activity in the specification, and no requirement that any particular proteins be phosphorylated.

As discussed in section 1, stimulation of protein tyrosine phosphorylation is a characteristic functional property of interferons. Applicants contend that the specification clearly discloses that the IFN-L polypeptides of the invention possess this characteristic functional property. Applicants have amended claims 1-3 to replace the limitation “has an activity of the polypeptide set forth in SEQ ID NO: 5,” with the requirement that the claimed IFN-L polypeptides exhibit the characteristic interferon activity of increasing cellular protein tyrosine phosphorylation. Applicants contend that

the amended claims satisfy the requirements of 35 U.S.C. § 112, second paragraph, and therefore, respectfully request withdrawal of this rejection.

Applicants respectfully contend that rejections based on 35 U.S.C. § 112, second paragraph, have been overcome by amendment or traversed by argument, and request that the Examiner withdraw all rejections made on this basis.

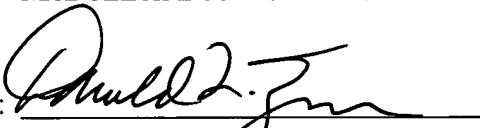
CONCLUSIONS

Applicants respectfully contend that all conditions of patentability are met in the pending claims as amended. Allowance of the claims is thereby respectfully solicited.

If Examiner Andres believes it to be helpful, she is invited to contact the undersigned representative by telephone at (312) 913-0001.

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff

Dated: November 13, 2002

By: 
Donald L. Zuhn, Ph.D.
Reg. No. 48,716

AMENDMENTS TO THE CLAIMS

Marked Up Versions of Amended Claims under 37 C.F.R. 1.121(c)(1)(ii)

1. (Amended Twice) An isolated nucleic acid molecule comprising a nucleotide sequence:
- (a) ~~the nucleotide sequence~~ as set forth in SEQ ID NO: 4;
 - (b) ~~the nucleotide sequence~~ of the DNA insert in ATCC Deposit No. PTA-976;
 - (c) ~~a nucleotide sequence~~ encoding the polypeptide as set forth in SEQ ID NO: 5;
 - (d) ~~a nucleotide sequence which that~~ hybridizes ~~under at least moderately conditions~~ to the complement of the nucleotide sequence of any of (a) - (c) under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences; or
 - (e) ~~a nucleotide sequence~~ complementary to the nucleotide sequence of any of (a) - ~~(e)~~(d).
2. (Amended Twice) An isolated nucleic acid molecule comprising:
- (a) a nucleotide sequence encoding a polypeptide which is at least about 70 percent identical to the polypeptide as set forth in SEQ ID NO: 5, wherein the encoded polypeptide ~~has an activity of the polypeptide set forth in SEQ ID NO: 5, upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation;~~
 - (b) a nucleotide sequence encoding an allelic variant of the nucleotide sequence as set forth in SEQ ID NO: 4, the nucleotide sequence of the DNA insert in ATCC Deposit No. PTA-976, or the nucleotide sequence of (a);
 - (c) a region of the nucleotide sequence of SEQ ID NO: 4, the DNA insert in ATCC Deposit No. PTA-976, or the nucleotide sequence of (a) or (b) encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide fragment ~~has an activity of the encoded polypeptide as set forth in SEQ ID NO: 5, upon exposure to mammalian cells, causes an increase in~~ cellular protein tyrosine phosphorylation, or is antigenic;
 - (d) a region of the nucleotide sequence of SEQ ID NO: 4, the nucleotide sequence of the DNA insert in ATCC Deposit No. PTA-976, or the nucleotide sequence of any of (a) - (c) comprising a fragment of at least about 16 nucleotides;

(e) a nucleotide sequence ~~which that~~ hybridizes ~~under at least moderately stringent conditions~~ to the complement of the nucleotide sequence of any of (a) - (d) under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences; or

(f) a nucleotide sequence complementary to the nucleotide sequence of any of (a) - ~~(d)~~(e).

3. (Amended Twice) An isolated nucleic acid molecule comprising a nucleotide sequence:

(a) ~~a nucleotide sequence~~ encoding a polypeptide as set forth in SEQ ID NO: 5 with at least one conservative amino acid substitution, wherein the encoded polypeptide ~~has an activity of the polypeptide set forth in SEQ ID NO: 5, upon exposure to mammalian cells, causes an increase in~~ cellular protein tyrosine phosphorylation;

(b) ~~a nucleotide sequence~~ encoding a polypeptide as set forth in SEQ ID NO: 5 with at least one amino acid insertion, wherein the encoded polypeptide ~~has an activity of the polypeptide set forth in SEQ ID NO: 5, upon exposure to mammalian cells, causes an increase in~~ cellular protein tyrosine phosphorylation;

(c) ~~a nucleotide sequence~~ encoding a polypeptide as set forth in SEQ ID NO: 5 with at least one amino acid deletion, wherein the encoded polypeptide ~~has an activity of the polypeptide set forth in SEQ ID NO: 5, upon exposure to mammalian cells, causes an increase in~~ cellular protein tyrosine phosphorylation;

(d) ~~a nucleotide sequence~~ encoding a polypeptide as set forth in SEQ ID NO: 5 which has a C- and/or N- terminal truncation, wherein the encoded polypeptide ~~has an activity of the polypeptide set forth in SEQ ID NO: 5, upon exposure to mammalian cells, causes an increase in~~ cellular protein tyrosine phosphorylation;

(e) ~~a nucleotide sequence~~ encoding a polypeptide as set forth in SEQ ID NO: 5 with at least one modification that is a conservative amino acid substitution, an amino acid insertion, an amino acid deletion, C-terminal truncation, or N-terminal truncation, wherein the encoded polypeptide ~~has an activity of the polypeptide set forth in SEQ ID NO: 5, upon exposure to~~ mammalian cells, causes an increase in cellular protein tyrosine phosphorylation;

(f) ~~a nucleotide sequence~~ of any of (a) - (e) comprising a fragment of at least about 16 nucleotides;

(g) ~~a nucleotide sequence which~~that hybridizes under at least moderately stringent conditions to the complement of the nucleotide sequence of any of (a) - (f) under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences; and or

(h) ~~a nucleotide sequence complementary to the nucleotide sequence of any of (a) - (e)(g).~~

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FORMAMIDE AND ITS USES IN MOLECULAR CLONING

Formamide is used as an ionizing solvent in aqueous buffers. Many batches of high-grade formamide are sufficiently pure to be used without further treatment. However, as a rule of thumb, if any yellow color is present or if there is even the hint of a smell of ammonia, the formamide should be purified. A more rigorous test of purity is to measure conductivity, which rises as the formamide breaks down to ammonium formate. The conductivity of pure formamide is 1.7 (Casey and Davidson 1977) and the conductivity of a 10^{-3} M solution of ammonium formate is $\sim 650 \mu\text{mho}$. The conductivity of formamide used in reannealing experiments should be $< 2.0 \mu\text{mho}$.

Formamide can be deionized by stirring for 1 hour on a magnetic stirrer with a mixed bed ion-exchange resin (e.g., Dowex AG8, Bio-Rad AG 501-X8, 20–50 mesh or X8[D]). The solution is then filtered through Whatman #1 paper and stored in small aliquots at -20°C , preferably under nitrogen. Each resin can be reused several times. X8(D) contains an indicator that changes color when the resin is exhausted.

Formamide is used in hybridization reactions, to resolve complex compressions in sequencing gels, and to denature DNA before electrophoresis as described below.

Resolving Compressions in Sequencing Gels

Including 25–50% (v/v) formamide in polyacrylamide sequencing gels destabilizes secondary structures in DNA and resolves some types of compression caused by anomalous migration of DNA bands (Brown 1984; Martin 1987). Gels containing formamide run slower and cooler than conventional polyacrylamide gels at the same voltage. It is usually necessary to increase the voltage by $\sim 10\%$ to maintain temperature. Gels containing formamide give fuzzier bands.

In addition to DNA sequencing, formamide is routinely included in gels used to analyze polymorphic (CA) repeats in mammalian DNAs. In the presence of formamide, the smear of bands that is generated during polymerase chain reaction (PCR) amplification of alleles is resolved into a discrete family whose members differ in size by 2 bp (Litt et al. 1993).

Denaturing RNA before Electrophoresis

Formamide (50%) is used to assist in denaturation of RNA before electrophoresis through denaturing formaldehyde-agarose gels (Lehrach et al. 1977).

Hybridization Reactions

Bonner et al. (1967) were the first to use formamide as a solvent in hybridization reactions. At the end of their brief paper, they wrote:

That formamide should take the place of elevated temperature in the hybridization process is to be expected. Aqueous solutions of formamide denature DNA as has been shown by Helmkamp and Ts'o (1961) and Marmur and T'so (1961). The concentrations of formamide required for DNA-RNA hybridization, 30–40 vol%, are well below the 60 vol% found by Marmur and T'so to be required for denaturation of native DNA (in 0.02 M NaCl–0.002 M sodium citrate).

What has now been found by serendipity is that hybridization as conducted in aqueous formamide possesses distinct advantages over hybridization conducted at elevated temperatures. These advantages include increased retention of immobilized DNA by the nitrocellulose filters and decreased nonspecific background absorption. These two factors combine to result in an increased reproducibility of replicates with the hybridization procedure. Hybridization in formamide solution at low temperature is helpful also in minimizing scission of nucleic acid molecules during prolonged periods of incubation.

In addition to these advantages, increased flexibility is introduced into the design of reaction conditions for a given experiment. It is more convenient to control this stringency of hybridization with formamide rather than through adjustment of the incubation temperature.

HYBRIDIZATION IN BUFFERS CONTAINING FORMAMIDE

Depression of the melting temperature (T_m) of duplex DNA is a linear function of the formamide concentration (McConaughy et al. 1969; Casey and Davidson 1977). For DNAs whose G+C content is in the range of 30–75%, the T_m is depressed by 0.63°C for each percentage of formamide in the hybridization mixture. Thus, the T_m of the hybrid formed between a probe and its target may be estimated from the following equation, which is modified from Bolton and McCarthy (1962):

$$T_m = 81.5^\circ\text{C} + 16.6 (\log_{10} [\text{Na}^+]) + 41 (\text{mole fraction } [\text{G}+\text{C}]) - 0.63 (\% \text{formamide}) - 500/n$$

where n is the length of the DNA in nucleotides. This equation applies to the reversible T_m defined by optical measurement of hyperchromicity at OD_{260} . The "irreversible" T_m (Hamaguchi and Geiduschek 1962), which is more important for autoradiographic detection of DNA hybrids, is usually 7–10°C higher than that predicted by the equation. Similar equations have been derived for RNA probes hybridizing to immobilized RNA (Bodkin and Knudson 1985):

$$T_m = 79.8^\circ\text{C} + 18.5 (\log_{10} [\text{Na}^+]) + 58.4 (\text{mole fraction } [\text{G}+\text{C}]) + 11.8 (\text{mole fraction } [\text{G}+\text{C}])^2 - 0.35 (\% \text{formamide}) - 820/n$$

and for DNA:RNA hybrids (Casey and Davidson 1977):

$$T_m = 79.8^\circ\text{C} + 18.5 (\log_{10} [\text{Na}^+]) + 58.4 (\text{mole fraction } [\text{G}+\text{C}]) + 11.8 (\text{mole fraction } [\text{G}+\text{C}])^2 - 0.50 (\% \text{formamide}) - 820/n$$

Comparison of these equations shows that the relative stability of nucleic acid hybrids in high concentrations of formamide decreases in the following order: RNA-RNA (most stable), RNA-DNA (less stable), and DNA-DNA (least stable). In 80% formamide, the T_m of an RNA-DNA hybrid is ~10°C higher than a DNA-DNA hybrid of equivalent base composition. It is therefore possible to find hybridization conditions that allow the formation of RNA-DNA hybrids and discourage the formation of DNA-DNA hybrids (Casey and Davidson 1977). This ability to suppress reannealing of DNA was extremely useful when S1 mapping of RNA was carried out with double-stranded DNA probes (Berk and Sharp 1977). However, the development of efficient methods to prepare single-stranded probes now allows annealing of RNA to DNA to be carried out under standard hybridization conditions without fear of competition from the complementary strand of DNA. As a consequence, the annealing conditions established by Casey and Davidson (1977) are today used only very rarely.

- The rate of DNA-DNA hybridization in 80% formamide is slower than in aqueous solution (Casey and Davidson 1977). Increasing the concentration of formamide decreases the rate of DNA-DNA renaturation by 1.1% for every 1% increase in the concentration of formamide (Hutton 1977). Therefore, the optimal rate in 50% formamide is 0.45 times the optimal rate in aqueous solution (Hutton 1977). In 80% formamide, the rate of DNA-DNA hybridization is three- to fourfold slower than in aqueous solution (Casey and Davidson 1977). This effect is a consequence of increased viscosity of the hybridization solution at the temperatures used for renaturation.
- The breakdown of formamide that occurs during prolonged incubation at temperatures in excess of 37°C can cause the pH of the hybridization buffer to drift upward (Casey and Davidson 1977). When formamide is included in the hybridization buffer, 6x SSPE is preferred to 6x SSC because of its greater buffering power.